Impacts of elevated ozone on growth and photosynthesis of *Metasequoia glyptostroboides* Hu et Cheng

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ABSTRACT

One-year-old *Metasequoia glyptostroboides* seedlings were exposed to non-filtered ambient air (NF) and elevated ozone (E-O₃, NF + 60 ppb) in open-top chambers for two years. E-O₃ accelerated leaf senescence, as indicated by significant decreases in photosynthetic pigment contents with the elongation of O₃ exposure. E-O₃ significantly affected gas exchange and carboxylation, inducing reductions in light-saturated photosynthesis (A_{sat}), the maximum activity of Rubisco ($V_{\text{c,max}}$) and the maximum electron transport rate (J_{max}). Chl a/b, $V_{\text{c,max}}/J_{\text{max}}$ and stomatal limitation (I) were not affected. Stomatal conductance (g_s) was significantly decreased by E-O₃ in the first year, but remained unchanged in the second year. It can be inferred that the decrease in A_{sat} by E-O₃ was mainly attributed to the changes in non-stomatal factors. After two years' exposure, E-O₃ caused significant decreases in canopy photosynthesis and leaf mass per area, and a significant increase in the number of branches, but induced slight, not significant decreases in growth and biomass. Therefore, it can be concluded that the carbon accumulation of the species *M. glyptostroboides* could be negatively affected after long-term exposure to high O₃ concentration.

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1. Introduction

Ground-level ozone (O₃) is considered to be the most relevant air pollutant causing acute and/or chronic damages to trees in the Northern Hemisphere, and in particular in East Asia [1–3], where a rapid increase in the emission of the main critical ozone precursors such as NO_x and volatile organic compounds (VOCs) has been observed. Due to increasing biogenic VOCs emissions over East China under the projected climate scenarios (2000–2050), increases in both surface O₃ concentration and high O₃ episodes are expected to occur [4]. Such current and future O₃ levels may pose a risk to subtropical tree species in this region.

Several studies have shown that, in northern temperate woody species, current and elevated O₃ concentrations induce significant reductions in photosynthesis [5,6] and shifts in carbon allocation [2,7]. For conifer or needle plants, O₃-induced reductions in photosynthesis have been reported for Norway spruce (*Picea abies*) [8,9] and different pine species such as ponderosa pine (*Pinus*

ponderosa) [10,11], Scots pine (*Pinus sylvestris*) [12,13] and loblolly pine (*Pinus taeda*) [14]. Among the various responses of tree species to O₃, decreases in chlorophyll content and in stomatal conductance (g_s), leading to a decline in photosynthesis, have been commonly observed [3,15]. However, in some cases, O₃-induced changes in g_s are insufficient to explain the decline in light-saturated photosynthesis (A_{sat}) [16]. Reductions in A_{sat} have been attributed to decreases in primary carboxylation enzyme Rubisco transcript levels, antioxidant contents and enzyme activity rather than changes in g_s [17,18]. A recent study suggests that elevated O₃ induces faster rates of change in photosynthesis rate compared to g_s because of the significant decrease in the maximum activity of Rubisco ($V_{\text{c,max}}$) and maximum electron transport rate (J_{max}), i.e., O₃ reduces the biochemical capacity to fix CO₂ [19].

The review of the effects of current and projected O₃ on biomass, growth, foliar physiology and biochemistry of trees using a meta-analysis approach concluded that current ambient O₃ concentration (40 ppb on average) significantly reduced the total biomass of trees by 7% compared with trees grown in charcoal-filtered (CF) air, while elevated O₃ (64 ppb) reduced total biomass by 11% compared with trees grown at current ambient O₃ concentration [20]. Furthermore, gymnosperms were less sensitive to than angiosperms [16], and the reduction in total biomass of gymnosperm species was largely driven by significant decreases in root

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Table 1

Daily 8-h (8:00–16:00) averaged O₃ concentration and AOT40 (the cumulative O₃ exposure over a threshold of the 1-h average [O₃] of 40 ppb) value for non-filtered (NF) and elevated ozone (E-O₃) treatments at two growing seasons.

Years	2009		2010	
	NF	E-O ₃	NF	E-O ₃
The maximum daily 8-h O ₃ (ppb)	81.5	169.6	73.2	136.7
The minimum daily 8-h O ₃ (ppb)	14.9	70.1	9.2	62.9
Daily 8-h mean (ppb)	37.0	54.6	34.9	53.9
AOT40 (ppm.h)	2.9	20.7	3.2	28.9

biomass [20]. Elevated O₃ can induce a shift in the resource allocation into height growth at the expense of diameter growth [9]. Furthermore, O₃ can also cause shift in carbon allocation away from roots [21,22], and often associated with decreased root/shoot ratio [23,24]. Unfortunately, there is little information on the biomass production and allocation of subtropical deciduous trees exposed to higher O₃ concentration.

Metasequoia glyptostroboides, commonly known as dawn redwood or Chinese redwood, is a “living fossil” discovered as a relict population in a remote area near the border of Sichuan in South-Central China in the 1940s [25]. In view of its esthetic beauty and economical value as a potential source of wood for forest products, this species with softy and feathery leaves has been widely naturalized for greening and ornamental plantings in cities from southeast China. The O₃ sensitivity of *M. glyptostroboides* has previously been studied in open top chambers, showing that acute O₃ exposure at both 100 and 200 ppb for 25 days (8 h per day fumigation) significantly reduced net photosynthetic rate, *g_s*, leaf length and crown width, compared to charcoal-filtered air (CF < 15 ppb) [26]. To extend further, this study investigated the pigment content, photosynthesis mechanism and growth of *M. glyptostroboides* in response to chronic O₃ exposure during two growing seasons. The aims of this study are (1) to assess whether elevated O₃ concentration in the near future will negatively affect foliar photosynthesis and growth in this species, and (2) to determine the mechanism explaining the observed ozone-induced reduction in *A_{sat}*, taking into account *g_s*, *V_{c,max}* and *J_{max}*.

2. Materials and methods

2.1. Experimental site and plant material

One-year-old seedlings of *M. glyptostroboides* were obtained from commercial nurseries and individually transplanted into 6 L circular plastic pots filled with native yellowish brown lateritic soil (soil organic C 0.98%, total N 1.36 g kg⁻¹, total P 0.27 g kg⁻¹, total K 1.41%) in mixture with litter collected under firry forest at the ratio of 1:1 (v/v). From November 2008 until the middle of April 2009, all seedlings were cultivated in a temperature controlled and double glazed greenhouse (25 ± 2 °C, air relative humidity 70–90%) in Ningbo, Zhejiang Province, China. After then, the seedlings with similar height and basal diameter were selected and moved to the experimental site which was located at the Tiantong National Field Observation and Research Station for Subtropical Forest Ecosystems (29°48' N, 121°47' E). At the beginning of the experiment, stem height and stem basal diameter in two directions at 5 cm of stem height were measured for each seedling. The saplings were about 40–50 cm tall and pre-adapted to open-top chambers (OTCs, octagonal base, 7 m² of growth space and 2.6 m in height) condition for two weeks before O₃ fumigation. During the cultivation and ozone exposure, plants were well watered with tap water to avoid drought stress.

Table 2

Analysis of variance of the effects of O₃ treatment, days after fumigation (DAF) and their interactions on the variables in *M. glyptostroboides* seedlings exposed to elevated O₃ concentration for two growing seasons.

	2009			2010		
	O ₃	DAF	O ₃ × DAF	O ₃	DAF	O ₃ × DAF
Total chl	<0.0001	<0.0001	0.004	<0.0001	<0.0001	0.012
Car	<0.0001	<0.0001	0.001	<0.0001	0.010	0.133
Chl a/b	0.163	<0.0001	0.587	0.699	<0.0001	0.191
<i>A_{sat}</i>	<0.0001	0.062	0.345	<0.0001	<0.0001	0.345
<i>g_s</i>	0.001	<0.0001	0.165	0.145	<0.0001	0.624
<i>C_i</i>	0.285	<0.0001	0.143	0.020	<0.0001	0.985
<i>V_{c,max}</i>	<0.0001	<0.0001	0.476	0.021	<0.0001	0.615
<i>J_{max}</i>	0.002	<0.0001	0.381	0.022	<0.0001	0.848
<i>V_{c,max}/J_{max}</i>	0.415	0.003	0.875	0.453	0.010	0.131
<i>l</i>	0.963	<0.0001	0.292	0.589	<0.0001	0.981

2.2. Fumigation

There were two O₃ treatments: non-filtered ambient air (NF) and NF + 60 ppb O₃ (E-O₃), with two chamber replicates per treatment. The selected seedlings were enclosed in OTCs, which were randomly used as control and O₃ treatment in the field. The averaged air velocity in each chamber corresponded to approximately two complete air exchange per minute. For E-O₃, O₃ was generated from pure oxygen by an electrical discharge O₃ generator (HY003, Chuangcheng Co. Jinan, China) and then mixed with ambient air to achieve the target O₃ concentration. The O₃ concentration in the OTCs was continuously monitored at approximately 10 cm above plant canopy using a UV absorption O₃ analyzer (Model 49i-Thermo, USA).

For each treatment, seedlings were transposed into two fumigation chambers with 10 plants per chamber. The position of plants was changed every 5–7 days within each OTC to eliminate positional effects. Every 10–15 days, the chambers were emptied and randomly reassigned to two target O₃ level, and the seedlings were replaced back according to their specified treatment levels. The fumigation periods were from 25 May to 10 September 2009, and 2 May to 12 September 2010 with a daily maximum of 8 h (from 08:00 to 16:00) when there was no rain, fog, mist or dew. There were totally 56 and 75 effective fumigation days during 2009 and 2010 growing seasons, respectively. During the fumigation period in 2010 growing season, mean air temperature and relative air humidity inside and outside the OTCs were 25.2 °C and 24.9 °C, and 81.8% and 88.2%, respectively, while the maximum air temperature and relative air humidity were 32.7 °C and 31.8 °C, and 99.0% and 93.6%, respectively.

Table 3

Height increments (cm), basal diameter increments (mm), branch increments (number), and carbon accumulation and distribution in *M. glyptostroboides* seedlings exposed to elevated O₃ concentration for two growing seasons.

	NF	E-O ₃	P value
Height increments (cm)	43.4 ± 8.3	40.6 ± 8.2	0.367
Basal diameter increments (mm)	5.54 ± 1.18	5.17 ± 1.35	0.427
Branch increments (number plant ⁻¹)	14.2 ± 5.6	21.5 ± 7.2	0.004
Total biomass (g plant ⁻¹)	144.47 ± 15.65	135.02 ± 19.44	0.538
Leaf mass (g plant ⁻¹)	32.75 ± 3.93	30.35 ± 5.80	0.280
Stem mass (g plant ⁻¹)	43.57 ± 4.77	41.36 ± 5.32	0.215
Root mass (g plant ⁻¹)	68.15 ± 8.65	63.30 ± 11.33	0.373
Root/above-ground	0.892 ± 0.061	0.885 ± 0.134	0.823

Fig. 1. Total chlorophyll (Chl), carotenoid (Car) content and the ratio of chlorophyll a/b (Chl a/b) in *M. glyptostroboides* seedlings exposed to non-filtered air (NF) and elevated ozone (E-O₃) treatments for two growing seasons (2009 and 2010). At each measurement day, statistically significant differences between O₃ treatments for each growing season are noted with an asterisk at $P < 0.05$. Error bars represent SD of the means ($N = 8$).

2.3. Leaf gas exchange and canopy photosynthesis measurements

Gas exchange was measured on fully expanded leaves from the upper part of the canopy with a portable photosynthesis system fitted with a red/blue LED light source (LI-6400, LI-COR Inc. Lincoln, NE, USA). The system controlled saturating PPFD (photosynthetic photon flux density) at $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$. The block temperature was set to the ambient average of $33 \pm 0.5^\circ\text{C}$, CO_2 at $380 \mu\text{mol mol}^{-1}$, and relative humidity (RH) at 50–65%. All measurements were conducted during 09:00–11:00 and recorded when the stability, or steady state indicated by total coefficient of variation, was $\leq 3\%$. Since the leaf was not large enough to fill in the red/blue LED light source chamber, the enclosed leaf was cut and its area was measured with a Li-Cor 3000 leaf area meter (Li-Cor, Lincoln, NE, USA), just after gas exchange measurement.

During the experiment, A/C_i curves were performed on upper leaves of two different plants per OTC with two OTCs replicates using the automatic program in the LI-6400 photosynthesis system. When g_s reached equilibrium, subsequent measurements were made following step-wise changes in reference CO_2

concentration: 380, 300, 200, 100, 50, 150, 250, 400, 600, 900, 1200, $1500 \mu\text{mol mol}^{-1}$, under PPFD of $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$, block temperature of $32 \pm 0.5^\circ\text{C}$ and relative humidity of 50–70%. $V_{c,\text{max}}$ and J_{max} and were derived by iteratively fitting curves to A/C_i response data according to the program of Sharkey et al. [27], as described by Long and Bernacchi [28]. Stomatal limitation (l) was calculated as $l = 1 - A_{C_a380}/A_{C_i380}$, where A_{C_a380} and A_{C_i380} represent net CO_2 assimilation rate at $C_a = 380 \text{ ppm}$ and at $C_i = 380 \text{ ppm}$, respectively.

Canopy photosynthesis (CP) was measured at the end of fumigation using a system consisting of a chamber, CO_2 analyzers (Li-840 $\text{CO}_2/\text{H}_2\text{O}$, Li-Cor, Inc, USA), air pump, desiccant and filter. The chamber was framed by aluminum pipes (40 cm $\times$ 40 cm square base, 1.2 m in height) and covered with transparent plastic film. During the measurements, the chamber was placed on a square base through water to make the system airtight. And the air inside the chamber was homogenized by two fans installed inside at 60 cm above the base. When the air CO_2 concentration showed a steady state and reached 500 ppm, the CO_2 value, air temperature (T) and relative humidity inside the chamber were monitored and recorded for 10 min at 10 s intervals. Just a few days after the measurement

Fig. 2. Light-saturated photosynthetic rate (A_{sat}), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) in *M. glyptostroboides* seedlings exposed to non-filtered air (NF) and elevated ozone (E- O_3) treatments for two growing seasons (2009 and 2010). At each measurement day, statistically significant differences between O_3 treatments for each growing season are noted with an asterisk at $P < 0.05$. Error bars represent SD of the means ($N = 8$).

of canopy photosynthesis, all leaves of the plant were harvested to determine the biomass and leaf area.

The canopy photosynthesis was calculated as:

$$\text{CP} = \left(\frac{\Delta c}{\Delta t} \right) \times \left(\frac{V}{22.4} \right) \times \left[\frac{273/(273 + T)}{S} \right]$$

where CP is canopy photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$), Δt is 10 s intervals, Δc is the decreased value of CO_2 concentration during 10 s intervals ($\mu\text{mol mol}^{-1}$), V is volume in the chamber (m^3); S is canopy leaf area (m^2), and T is air temperature inside chamber ($^{\circ}\text{C}$).

2.4. Pigments content

About 0.1 g of leaves from three plants from each OTC were extracted with 4 ml 95% ethanol in the dark for 48 h at 4°C . The absorbance of leaf pigment extracts was measured at 646, 663 nm and 470 nm. Total chlorophyll (Chl) and carotenoid (Car) content were calculated according to the specific absorption coefficients provided by Lichtenthaler [29].

2.5. Growth and biomass production

At the end of the experiment, the area of 30 unwrinkled and undamaged leaves per plant was measured with a Li-Cor 3000 leaf area meter, and dry mass was determined after oven-drying at 80°C until a constant weight in order to calculate leaf mass per area (LMA). All seedlings were harvested and separated into leaves, stems and roots. Dry mass was determined after oven-drying at 80°C until a constant weight. The canopy leaf area was calculated as $S = \text{leaf mass}/\text{LMA}$.

2.6. Statistical analysis

In order to test the influence of chamber on measured variables, all data were firstly checked for normal distribution and homogeneity of variance, and then subjected to multivariate analysis of variance (MANOVA) using SPSS 16.0 software package for Windows. Since chamber-to-chamber variation within treatments was not significant, data for each dependent variable were subjected to the analysis of variance with mixed linear model (GLM) to test the effects of O_3 , exposure days and their interactions by Tukey's

Fig. 3. The maximum velocity of carboxylation ($V_{c,max}$), maximum rate of electron transport (J_{max}), the ratio of $V_{c,max}/J_{max}$, and stomatal limitation to photosynthesis (l) in *M. glyptostroboides* seedlings exposed to non-filtered air (NF) and elevated ozone (E-O₃) treatments for two growing seasons (2009 and 2010). At each measurement day, statistically significant differences between O₃ treatments for each growing season are noted with an asterisk at $P < 0.05$. Error bars represent SD of the means ($N = 4$).

HSD test, with eight or four plants as independent experimental units for each treatment. Student's *t* test was used to compare the means between NF and E-O₃ on individual measurement dates if there was significant O₃ or interaction effect. Treatment effects were considered significant if $P < 0.05$.

3. Results

Based on seasonal 8-h average O₃ concentration (M8), the seedlings experienced similar O₃ level in E-O₃ during both

growing seasons (Table 1). The O₃ concentration in E-O₃ was 48% and 54% higher than that in NF in 2009 and 2010, respectively. E-O₃ showed higher AOT40 values (AOT40, the cumulative O₃ exposure over a threshold of the 1-h average [O₃] of 40 ppb) in 2010 than in 2009 because the fumigation period in the former year was longer.

Statistical analysis indicated that E-O₃ significantly reduced Chl and Car contents ($P < 0.001$) in both growing seasons, but it has no significant effect on Chl a/b (Table 2). In the first year, significant reductions in Chl and Car contents by E-O₃ were observed after 60 days of fumigation (DAF), with an average decrease of 25% and

Fig. 4. Canopy photosynthesis (CP) and leaf mass per area (LMA) in *M. glyptostroboides* seedlings exposed to non-filtered air (NF) and elevated ozone (E-O₃) treatments for two growing seasons (2009 and 2010). Statistically significant differences between O₃ treatments for each growing season are noted with an asterisk at $P < 0.05$. Error bars represent SD of the means ($N = 4$).

11% during the whole growing season, respectively (Fig. 1a and b). During the second year, similar reductions in Chl and Car contents (by 25% and 15% on average, respectively) were also observed in the E-O₃ treatment (Fig. 1d and e).

ANOVA results indicated that E-O₃ significantly reduced A_{sat} in both growing seasons, and affected C_i only at the second year (Table 2). In the first year, E-O₃ significantly decreased A_{sat} by 17%, 31% and 14% at 35, 90, and 110 DAF, respectively, compared to NF (Fig. 2a). Furthermore, E-O₃ significantly decreased g_s by 28% and 22% at 90 and 110 DAF, respectively (Fig. 2b). In the second year, E-O₃ significantly decreased A_{sat} since 60 DAF ($P < 0.05$), with a maximum decrease of 26% and 18% for A_{sat} , respectively, compared to NF (Fig. 2d). On the other hand, C_i was not affected by E-O₃ at any measurement time, although a slight non-significant increase was observed across four measurements in the second year (Fig. 2f).

E-O₃ significantly impaired $V_{\text{c,max}}$ and J_{max} (Table 2), as indicated that a magnitude of decrease increased progressively each year as leaves aged (Fig. 3). However, E-O₃ had no effects on $V_{\text{c,max}}/J_{\text{max}}$ and stomatal limitation (l) in both growing seasons (Fig. 3c, d, g, h). In the first year, at 90 DAF, E-O₃ significantly decreased $V_{\text{c,max}}$ and J_{max} by 37% and 40%, respectively (Fig. 3a and b). In the second year, similar negative effects on $V_{\text{c,max}}$ and J_{max} were observed: $V_{\text{c,max}}$ decreased significantly by 27% and J_{max} by 24% (Fig. 3e and f).

In 2009, E-O₃ had no significant effects on CP and LMA (Fig. 4). However, after two years of O₃ fumigation, CP and LMA decreased significantly by 21% and 5%, respectively, in E-O₃. E-O₃ caused slight, not significant decreases in height and basal diameter increments, whereas the number of branches significantly increased ($P < 0.05$, Table 3). Furthermore, E-O₃ had no significant effects on

leaf, stem, root and total biomass and thus the ratio of root and above-ground biomass (Table 3).

4. Discussion

Further increases in ground-level O₃ concentration is predicted in East Asian regions due to rapid economic growth. Several studies on native species in Asia reported that O₃ caused visible leaf injury, reduced carbon assimilation, and limited the growth of trees (e.g., *Cyclobalanopsis glauca* and *Fagus crenata* [30,31]). In this study, E-O₃ with M8 of 54 ppb significantly caused changes in some photosynthetic parameters at leaf-level and canopy-level, e.g. pigment contents and net photosynthetic rate in both growing seasons, while two years' O₃ fumigation did not affect biomass and C assimilate distribution of *M. glyptostroboides*. From the changes in these physiological parameters, it implied that two years' O₃ fumigation significantly decreased the photosynthetic carbon gain at whole-plant level. This can be largely attributed to significant decreases in photosynthesis rate and pigments contents at leaf level rather than to changes in the total area of green leaves (LMA and leaf biomass to E-O₃).

In general, chronic O₃ exposure resulted in stomatal closure, which is considered as the most important avoidance mechanisms to O₃ stress [17,32] because a decrease in g_s protects trees from air pollution by decreasing O₃ uptake [33]. In fact, in this study, leaves exhibited a significant decrease in g_s in response to E-O₃ only at the end of the first growing season (Fig. 2). This is in agreement with a previous study on *Arbutus unedo*, a Mediterranean evergreen in which O₃ did not significantly reduce midday steady-state g_s compared to controls, but it slowed stomatal closure [34]. In addition, the response of g_s to E-O₃ differed between the two growing seasons, while g_s considerably reduced in 2009, it remained unchanged in 2010 (Table 2 and Fig. 2). The O₃ exposure induces an uncoupling of photosynthesis and stomatal conductance, as previously observed in three native tree species from China (*Ailanthus altissima*, *Fraxinus chinensis* and *Platanus orientalis*) [35]. Lombardozzi et al. [19] reported that the typical coupling of stomatal conductance and photosynthesis does not always hold under high O₃ conditions, because O₃ can directly affect the function of stomatal cells so that g_s no longer responds dynamically to changes in C_i and the rate of carboxylation due to enzyme and membrane damage. Unchanged g_s with increased C_i in the second growing season, as observed in this study, indicated that the decrease in photosynthesis was independent of g_s [15,36].

One possible explanation to the decreased net photosynthetic rate is related to the significant decreases in leaf-level $V_{\text{c,max}}$ and J_{max} caused by E-O₃. Watanabe et al. [31] demonstrated that O₃ significantly reduced the $V_{\text{c,max}}$ and J_{max} in leaves of beech, but stomatal limitation of photosynthesis was unaffected by O₃. Similar results were also found in some deciduous and evergreen species such as *Liriodendron chinense* and *Cinnamomum camphora* [17,37]. On the other hand, unchanged l accompanied by a decrease in pigments content during the two-year study further confirmed that O₃-induced the photosynthesis down-regulation was due to non-stomatal factors rather than stomatal closure. From the information above, the conifer species showed the similar mechanism on the reduced photosynthesis rate by high O₃ concentration with evergreen or deciduous broadleaf species.

However, E-O₃ with AOT40 of 50 ppm h (the sum of two seasons) had no significant effects on stem height and diameter increments, biomass accumulation and carbon distribution within plants, in spite of the significant decrease in canopy photosynthetic rate after two years fumigation (Table 3). These results suggest that whole

plant growth could be negatively affected after a long-term O₃ exposure.

M. glyptostroboides has an inherently LMA with an average value of 60 g m⁻², similar to those O₃-sensitive deciduous tree species [38], suggesting that *M. glyptostroboides* could be sensitive to O₃. Bussotti [39] has pointed out that LMA was a better parameter to distinguish O₃ sensitivity among species, and those species with some ecological characteristics such as low LMA and short leaf lifespan, high gas exchange rate and rapid growth rate, and favorable habitats are more sensitive to O₃. In this study, the different response to E-O₃ between leaves and whole plants can be explained by short duration of effective fumigation (56 and 75 days in 2009 and 2010, respectively) and low O₃ uptake due to partial stomatal closure at relatively high vapour pressure deficit (VPD) and temperature in typical summer days of subtropical climate. Moreover, E-O₃ significantly increased the number of branches but not the stem biomass, suggesting that the length of branch could be reduced after O₃ exposure (Table 3).

Overall, the results indicated that non-stomatal factors including decreased Chl and Car contents, $V_{c,max}$ and J_{max} accounted for the decrease in A_{sat} . Moreover, *M. glyptostroboides* apparently exhibited evident phenotypic plasticity, and similar decreases in root and above-ground in response to elevated O₃ concentration, suggesting that root growth is as susceptible to O₃ as the above-ground part.

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